

This listing of the claims will replace all prior versions and listings of the claims in this application.

**In the Claims:**

1. (Amended) A method of treating disturbances in iron distribution in a patient suffering from non insulin dependent diabetes mellitus comprising administering a therapeutically effective amount of human erythropoietin protein having the amino acid sequence of SEQ ID NO:1.
2. (Cancelled)
3. (CANCELLED) The method of claim 1, wherein the erythropoietin protein is epoetin alfa or epoetin beta.
4. (CANCELLED) The method of claim 1, wherein the erythropoietin protein has the amino acid sequence of SEQ ID NO:1.
5. (Amended) The method of claim 1, wherein the erythropoietin protein has the sequence of human erythropoietin A method of treating disturbances in iron distribution in a patient suffering from non insulin dependent diabetes mellitus comprising administering a therapeutically effective amount of human erythropoietin protein having the amino acid sequence of SEQ ID NO:1 modified by the addition of from 1 to 6 up to 3 glycosylation sites, wherein the modification is selected from the group consisting of:  
Asn<sup>30</sup>Thr<sup>32</sup>,  
Asn<sup>51</sup>Thr<sup>53</sup>,  
Asn<sup>57</sup>Thr<sup>59</sup>,  
Asn<sup>69</sup>,  
Asn<sup>69</sup>Thr<sup>71</sup>,  
Ser<sup>68</sup>Asn<sup>69</sup>Thr<sup>71</sup>.

Val<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup>.  
Ser<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup>.  
Ser<sup>87</sup>Asn<sup>88</sup>Gly<sup>89</sup>Thr<sup>90</sup>. (SEQ ID NO: 3);  
Ser<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup>Thr<sup>92</sup>.  
Ser<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup>Ala<sup>162</sup>.  
Asn<sup>69</sup>Thr<sup>71</sup>Ser<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup>.  
Asn<sup>30</sup>Thr<sup>32</sup>Val<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup>.  
Asn<sup>89</sup>Ile<sup>90</sup>Thr<sup>91</sup>.  
Ser<sup>87</sup>Asn<sup>89</sup>Ile<sup>90</sup>Thr<sup>91</sup>.  
Asn<sup>136</sup>Thr<sup>138</sup>.  
Asn<sup>138</sup>Thr<sup>140</sup>.  
Thr<sup>125</sup>, and  
Pro<sup>124</sup>Thr<sup>125</sup>.

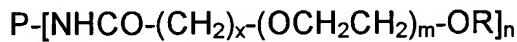
6. (Amended) The method of claim 1, wherein the erythropoietin protein is darbepoetin alfa. A method of treating disturbances in iron distribution in a patient suffering from non insulin dependent diabetes mellitus comprising administering a therapeutically effective amount of human erythropoietin protein, without administering iron, wherein the protein (EPO) is an analog of SEQ ID NO:1, said analog is selected from the group consisting of: (a) human erythropoietin protein having the amino acid sequence, Ser Ser Ser Ser Lys Ala Pro Pro Ser Leu Pro Ser Pro Ser Arg Leu Pro Gly Pro Ser Asp Thr Pro Ile Leu Pro Gln (SEQ ID NO: 4), extending from the carboxy terminus; (b) the analog in (a) further comprising Ser<sup>87</sup> Asn<sup>88</sup> Thr<sup>90</sup> EPO; (c) the analog in (a) further comprising Asn<sup>30</sup> Thr<sup>32</sup> Val<sup>87</sup> Asn<sup>88</sup> Thr<sup>90</sup> EPO; (d) Gln<sup>24</sup> Ser<sup>87</sup> Asn<sup>88</sup> Thr<sup>90</sup> EPO; (e) Gln<sup>38</sup> Ser<sup>87</sup> Asn<sup>88</sup> Thr<sup>90</sup> EPO; (f) Gln<sup>83</sup> Ser<sup>87</sup> Asn<sup>88</sup> Thr<sup>90</sup> EPO and (g) darbepoetin alfa.

7. (Original) The method of claim 1, wherein the erythropoietin protein is pegylated.

8. (Amended) The method of claim 7, wherein the erythropoietin protein is a conjugate A method of treating disturbances in iron distribution in a patient suffering from non-insulin dependent diabetes mellitus comprising administering a conjugate of human erythropoietin protein of SEQ ID NO:1, wherein said conjugate comprising an comprising the erythropoietin protein of SEQ ID NO:1 having at least one to three free amino groups and having the *in vivo* biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells and selected from the group consisting of human erythropoietin and analogs thereof which have a sequence of human erythropoietin modified by the addition of from 1 to 6 glycosylation sites or a rearrangement of at least one glycosylation site; said erythropoietin protein being covalently linked to n poly(ethylene glycol) groups of the formula  $-\text{CO}-(\text{CH}_2)_x-$   $(\text{OCH}_2\text{CH}_2)_m-\text{OR}$  with the  $-\text{CO}$  of each poly(ethylene glycol) group forming an amide bond with one of said amino groups; wherein R is a lower-alkyl; x is 2 or 3; m is from about 450 to about 900; n is from 1 to 3; and n and m are chosen so that the molecular weight of the conjugate minus the erythropoietin protein is from 20 kilodaltons to 100 kilodaltons.

9. (Original) The method of claim 8, wherein x is 3, m is 650 to 750, n is 1 and R is methyl.

10. (Original) The method of claim 8 wherein the conjugate has the formula

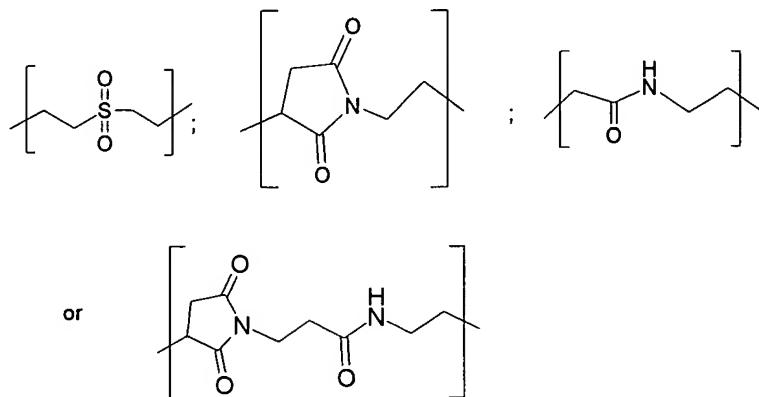


wherein P is the residue of the protein without the free amino group that forms the amide linkage;

- R is lower alkyl;
- x is 2 or 3;
- m is from about 450 to about 900;
- n is from 1-3; and

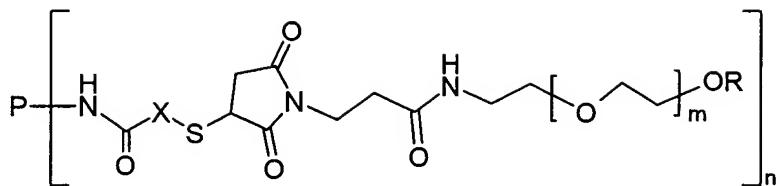
wherein m and n are selected such that the molecular weight of the conjugate minus the erythropoietin protein is from about 20 kD to about 100 kD.

11. (Amended) The method of claim 7, wherein the erythropoietin protein is a conjugate, said conjugate comprising an erythropoietin protein having at least one free amino group and having the *in vivo* biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells and selected from the group consisting of human erythropoietin protein and analogs thereof which have the primary structure of human erythropoietin protein modified by the addition of from 1 to 6 glycosylation sites; said erythropoietin protein being covalently linked to from one to three lower alkoxy poly(ethylene glycol) groups, each poly(ethylene glycol) group being A method of treating disturbances in iron distribution in a patient suffering from non-insulin dependent diabetes mellitus comprising administering a conjugate of human erythropoietin of SEQ ID NO:1 wherein, said conjugate comprises the erythropoietin protein of SEQ ID NO:1 having one to three free covalently linked to the erythropoietin protein via a linker of the formula  $-C(O)-X-S-Y-$  with the C(O) of the linker forming an amide bond with one of said amino groups, X is  $-(CH_2)_k-$  or  $-CH_2(O-CH_2-CH_2)_k-$ , k is from 1 to 10, Y is



the average molecular weight of each poly(ethylene glycol) moiety is from about 20 kilodaltons to about 40 kilodaltons, and the molecular weight of the conjugate is from about 51 kilodaltons to about 175 kilodaltons.

12. (Amended) The method of claim 11, wherein the erythropoietin conjugate has the formula:



wherein n is an integer from 1 to 3; m is an integer from 450 to 900; R is lower-alkyl; X is  $-(CH_2)_k-$  or  $-CH_2(O-CH_2-CH_2)_k-$ , k is 1 to 10 and P is the residue of the erythropoietin protein without the n amino groups which form an amide linkage with X.

13. (Amended) A pharmaceutical composition for the treatment of disturbances in iron distribution comprising from about 25 to about 2,500  $\mu$ g/ml of erythropoietin protein, from about 10 to about 200 mmol/l sulfate and having a pH of from about 6.0 to about 7.0.

14. (Amended) The pharmaceutical composition of claim 13 comprising from about 50 to about 2,500  $\mu$ g/ml of erythropoietin protein, 10 mM sodium phosphate, 40 mM sodium sulfate, 3% mannitol (w/v), 10 mM methionine and 0.01% poloxamer 188 (w/v) and has a pH of about 6.2.

15. (Amended) The pharmaceutical composition of claim 13 comprising from about 50 to about 2,500  $\mu$ g/ml of erythropoietin protein, 40 mM arginine, 30 mM sodium sulfate, 3% mannitol (w/v), 10 mM methionine, 0.01% poloxamer 188 (w/v) and having a pH of about 6.2.

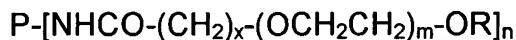
16. (Amended) A method of treating disturbances in iron distribution in a patient suffering from diabetes comprising administering a therapeutically effective amount of a pharmaceutical composition of human erythropoietin protein of SEQ ID NO:1, wherein the pharmaceutical composition comprises from about 25 to about 2,500  $\mu$ g/ml of erythropoietin protein, from about 10 to about 200 mmol/l sulfate and having a pH of from about 6.0 to about 7.0.

17. (CANCELLED) The method of claim 1, wherein the erythropoietin protein is epoetin alfa or epoetin beta.
18. (CANCELLED) The method of claim 1, wherein the erythropoietin protein has the amino acid sequence of SEQ ID NO:1.
19. (Amended) ~~The method of claim 116, wherein the erythropoietin protein has the sequence of human erythropoietin~~ A method of treating disturbances in iron distribution in a patient suffering from diabetes comprising administering a therapeutically effective amount of a composition of human erythropoietin protein of SEQ ID NO:1 modified by the addition of from 1 to 6 up to 3 glycosylation sites, wherein the composition comprises from about 25 to about 2,500 µg/ml of erythropoietin protein, from about 10 to about 200 mmol/l sulfate and having a pH of from about 6.0 to about 7.0.
20. (CANCELLED) The method of claim 1, wherein the erythropoietin protein is darbepoetin alfa.
21. (Amended) The method of claim 4 16, wherein the erythropoietin protein is pegylated.
22. (Amended) ~~The method of claim 7, wherein the erythropoietin protein is a conjugate~~ A method of treating disturbances in iron distribution in a patient suffering from diabetes comprising administering a composition of a conjugate of human erythropoietin protein of SEQ ID NO:1, wherein said conjugate comprising an comprises the erythropoietin protein of SEQ ID NO:1 having at least one to three free amino groups and having the *in vivo* biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells and selected from the group consisting of human erythropoietin and analogs thereof which have a sequence of human erythropoietin modified by the addition of from 1 to 6 glycosylation sites or a rearrangement of at least one glycosylation site; said erythropoietin protein being

covalently linked to n poly(ethylene glycol) groups of the formula  $-\text{CO}-(\text{CH}_2)_x-(\text{OCH}_2\text{CH}_2)_m-\text{OR}$  with the  $-\text{CO}$  of each poly(ethylene glycol) group forming an amide bond with one of said amino groups; wherein R is a lower-alkyl; x is 2 or 3; m is from about 450 to about 900; n is from 1 to 3; and n and m are chosen so that the molecular weight of the conjugate minus the erythropoietin protein is from 20 kilodaltons to 100 kilodaltons, wherein said composition comprises from about 25 to about 2500 ug/ml of erythropoietin protein, from about 10 to about 200 mmol/l sulphate and having a pH of from about 6.0 to about 7.0.

23. (Amended) The method of claim 8 22, wherein x is 3, m is 650 to 750, n is 1 and R is methyl.

24. (Amended) The method of claim 8 22, wherein the conjugate has the formula



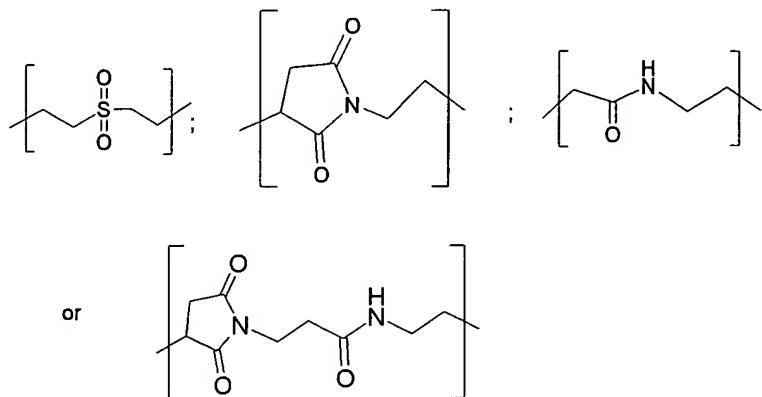
wherein P is the residue of the protein without the free amino group that forms the amide linkage;

- R is lower alkyl;
- x is 2 or 3;
- m is from about 450 to about 900;
- n is from 1-3; and

wherein m and n are selected such that the molecular weight of the conjugate minus the erythropoietin protein is from about 20 kD to about 100 kD.

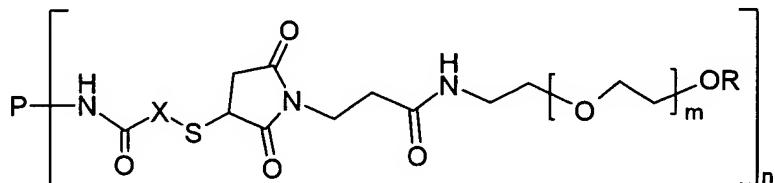
25. (Amended) The method of claim 7 A method of treating disturbances in iron distribution in a patient suffering from diabetes comprising administering a composition of a conjugate of human erythropoietin protein of SEQ ID NO:1, wherein the erythropoietin protein is a conjugate, said conjugate comprising an comprising the erythropoietin protein of SEQ ID NO:1 having at least one to three free amino groups and having the *in vivo* biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells and selected from the group consisting of

human erythropoietin protein and analogs thereof which have the primary structure of human erythropoietin protein modified by the addition of from 1 to 6 glycosylation sites; said erythropoietin protein being covalently linked to from one to three lower-alkoxy poly(ethylene glycol) groups, each poly(ethylene glycol) group being covalently linked to the erythropoietin protein via a linker of the formula  $-\text{C}(\text{O})-\text{X}-\text{S}-\text{Y}-$  with the  $\text{C}(\text{O})$  of the linker forming an amide bond with one of said amino groups,  $\text{X}$  is  $-(\text{CH}_2)_k-$  or  $-\text{CH}_2(\text{O}-\text{CH}_2-\text{CH}_2)_k-$ ,  $k$  is from 1 to 10,  $\text{Y}$  is



the average molecular weight of each poly(ethylene glycol) moiety is from about 20 kilodaltons to about 40 kilodaltons, and the molecular weight of the conjugate is from about 51 kilodaltons to about 175 kilodaltons wherein said composition comprises from about 25 to about 2500 ug/ml of erythropoietin protein, from about 10 to about 200 mol/l sulfate and having a pH of from about 6.0 to about 7.0.

26. (Amended) The method of claim 4125, wherein the erythropoietin-conjugate has the formula:



wherein n is an integer from 1 to 3; m is an integer from 450 to 900; R is lower-alkyl; X is  $-(CH_2)_k-$  or  $-CH_2(O-CH_2-CH_2)_k-$ , k is 1 to 10 and P is the residue of the erythropoietin protein without the n amino groups which form an amide linkage with X.